RENILLA LUCIFERASE ASSAY

This protocol is adapted from Promega's Renilla Luciferase Assay System Protocol by the Gene Expression Lab.
This protocol is for use with Promega's Renilla Luciferase Assay Systems. For additional technical inquiries, contact Technical Service at 800-356-9526 or www.promega.com

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BEFORE STARTING THE EXPERIMENT
ASSAY PROTOCOL

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1. Prepare the Renilla Luciferase Assay Lysis Buffer
    - Renilla Luciferase Assay Lysis Buffer is supplied as a 5X concentrate. Prepare a sufficient quantity of the 1X working solution by adding 1 volume of 5X Renilla Luciferase Lysis Buffer to 4 volumes of distilled water and mixing well.
    - The diluted (1X) Lysis Buffer may be stored at 4°C for up to one month. However, we recommend that Lysis Buffer be prepared fresh in the amount needed for each experiment. Store the 5X RenillaLuciferase Assay Lysis Buffer at –20°C.
2. Prepare the Renilla Luciferase Assay Reagent.
    - Take 1 volume of 100X Renilla Luciferase Substrate to 100volumes of Renilla Luciferase Assay Buffer
    - (i.e. For 10 Assays: 10 µL of 100x Renilla Luciferase Substrate in 1mL of Renilla Luciferase Assay Reagent).
3. Preparation of cells cultured in multi-well plates
    - This step is described in our Lentivirus Transfection protocol or it can be found in the Renilla Luciferase Assay System manual at http://www.promega.com/tbs/tm055/tm055.pdf

Process

4. Add 100 µL of Renilla Luciferase Assay Reagent to the luminometer tube.
5. Add 20 µL of cell lysate. Mix quickly by flicking the tube with a finger or vortex to thoroughly mix (1–2 seconds).
    - We usually have to dilute our cell lysate into a total volume of 20 µL with the 1x RLA. Generally we use 1-2 µL of cell lysate, diluting to 20 µL total volume.
6. Place tube in luminometer and initiate measurement. Results are immediately transferred into excel data table from luminometer.

- To blank the luminometer – add 100 µL of Renilla Luciferase Assay Reagent to the tube and add 20 µL of 1x Renilla Luciferase Assay Lysis Buffer as a blank measurement and place in luminometer. This RLU reading can be used as a background measurement for the assay.
